Support for the Amendments

Amended Claims

Support for the amendment to claim 5 is found throughout the specification, for example: at page 13, lines 20-22; at page 58, lines 20-23; and at page 59, lines 8-10.

Amended Sequence Listing

As required by 37 C.F.R. § 1.821(d), enclosed is an amended sequence listing consisting of 13 pages. Applicants have also provided both a paper and CRF copy of the corrected sequence listing.

Please note that Applicants' have added two additional sequence identifiers, SEQ ID NO.s:30 and 31. New SEQ ID NO.s:30 and 31 contain ced-4 sequences 1 and 2, respectively, from Figure 3. SEQ ID NO.s:1-28 were amended to add organism names. Applicants have also amended the amino acid sequence of SEQ ID NO.:8 to contain the second ICaBP sequence from Figure 3, and amended SEQ ID NO.:23 to include the sequence found at page 38, line 15. SEQ ID NO.s:23-28 were renumbered to reflect this insertion; original SEQ ID NO.s:23-28 are now SEQ ID NO.s:24-29, respectively.

Summary of the Interview

On November 4, 2001 the Examiner and the Applicant discussed discrepancies between the claim numbers cited in the Office Action and in the claims. Applicant requested a new Office Action. Examiner provided a new Office Action that was mailed on November 16, 2001.

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Summary of the Office Action

Claims 5, 6, 16, and 19 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, first paragraph. Claims 5, 6, 16, and 19 stand further rejected under 35 U.S.C. § 112, second paragraph. Claims 5, 16, and 19 stand further rejected under 35 U.S.C. § 102(b) or 103(a).

Rejections under 35 U.S.C. § 112, first paragraph

Claims 5, 6, 16, and 19 are rejected under 35 U.S.C. § 112, first paragraph, based on the assertion that the specification does not provide enablement for an isolated protein encoded by any and all *ced-3* nucleic acids. Applicants disagree, particularly in view of the foregoing amendments to claims 5 and 6.

The Examiner bases this rejection on the following assertions: (1) that Applicants fail to provide sufficient guidance as to the characteristics of a *ced-3* gene or protein; (2) that Applicants disclose only the sequence of *C. elegans ced-3* nucleic acid (SEQ ID NO.:18) and protein (SEQ ID NO.:19); (3) that Applicants fail to disclose whether changes in SEQ ID NO.:18 would encode an amino acid sequence that retains the function of SEQ ID NO.:19; (4) that Applicants fail to teach whether any and all organisms would have a *ced-3* gene; (5) that Applicants fail to disclose whether all Ced-3 proteins would fulfill the same function as *C. elegans ced-3*; and (6) that Applicants fail to provide a bioassay to determine the biological activity of Ced-3 related proteins.

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The first basis for rejection is that the specification fails to disclose the characteristics of a *ced-3* gene or protein. The Applicants direct the Examiner's attention to the specification (page 13, lines 15-20) where *ced-3* related genes are defined as those that have structural similarity to the nucleotide sequences of *ced-3* genomic DNA or cDNA, or whose encoded proteins have similarity to the amino acid sequence of the Ced-3 protein. The specification defines genes that are functionally related to *ced-3* as those that cause cell death (page 13, lines 20-25). In addition, the invention provides a *C. elegans* bioassay to measure this activity (page 17, lines 15-34, and pages 18-20).

The Examiner is concerned about the degree of similarity or identity required for a nucleic acid sequence to "qualify" as a *ced-3* nucleic acid, or for an amino acid sequence to "qualify" as a Ced-3 polypeptide. Applicants direct the Examiner's attention to claim 19 that recites a protein encoded by an isolated nucleic acid comprising both functional and structural similarities to the *ced-3* nucleic acid sequence, SEQ ID NO:18. The encoded polypeptide is hydrophilic in nature, contains a serine rich domain, and causes cell death in a bioassay. While Applicants have not called out a particular percentage of sequence identity, Applicants have required that Ced-3 related polypeptides exhibit sequence similarity and characteristic features associated with *C. elegans* Ced-3 protein, *i.e.,* a hydrophilic nature and a serine rich domain (page 58, lines 20-25), and that the nucleic acid encoding this polypeptide complements *ced-3 in vivo*. Clearly, Applicants have defined the characteristics that identify *ced-3* nucleic acids and polypeptides.

The second basis for rejection turns on the assertion that Applicants disclose only *C. elegans ced-3*. This is incorrect. Applicants direct the Examiner's attention to page

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59, lines 1-10, where Applicants disclose the isolation of *ced-3* genes from other nematodes, *C. briggsae* and *C. vulgaris*. The *ced-3* genes of *C. briggsae* and *C. vulgaris* were isolated using the methods provided within the specification, clearly demonstrating that the methods disclosed enable one skilled in the art to identify additional members of the *ced-3* gene family in other organisms.

A third basis for rejection turns on the assertion that the specification fails to disclose whether changes in the *ced-3* nucleotide or amino acid sequence would yield a functional *ced-3* gene or protein. The Applicants direct the Examiners attention to Figure 7 of the specification. Figure 7 is an alignment of the Ced-3 polypeptides encoded by the *ced-3* nucleic acid sequences identified in *C. elegans*, *C. briggsae*, and *C. vulgaris*. The specification provides an analysis of this alignment (page 59, lines 3-16), and a description and analysis of various mutations isolated in the *ced-3* gene,

> Sequence comparison of the three *ced-3* genes showed that the non-serine-rich region of the proteins is highly conserved. In *C. briggsae* and *C. vulgaris*, many amino acids in the serine-rich region are dissimilar compared to the C. elegans CED-3 protein (Figure 7). It seems that what is important in the serine-rich region is the overall serine-rich feature rather than the exact amino acid sequence.
>
> This hypothesis is also supported by analysis of *ced-3* mutations in *C. elegans*: none of the 12 EMS-induced mutations is in the serine-rich region, suggesting that mutations in this region might not affect the function of the Ced-3 protein and thus, could not be isolated in the screen for *ced-3* mutants. (Emphasis added)

Thus, the specification teaches a region of the protein, i.e. the serine-rich region, which may be changed without affecting protein function, and another region, i.e. the non-serine rich region, which may not be changed. Changes in the highly conserved non-serine-rich region impair Ced-3 function. Furthermore, Applicants determined the DNA

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sequences of twelve EMS-induced *ced-3* alleles, and analyzed the effect of these mutations (page 58, lines 2-17).

> Nine of the 12 [isolated *ced-3* mutations] are missense mutations. Two of the 12 are nonsense mutations, which might prematurely terminate the translation of *ced-3*. These nonsense *ced-3* mutants confirmed that the *ced-3* gene is not essential for viability. One of the 12 mutations is an alteration of a conserved splicing acceptor G, and another has a change of a 70% conserved C at the splice site, which could also generate a stop codon even if the splicing is correct. Interestingly, these EMS-induced mutations are in either the N-terminal quarter or C-terminal half of the protein. In fact, 9 of the 12 mutations occur within the region of *ced-3* that encodes the last 100 amino acids of the protein. Mutations are notably absent from the middle part of the ced-3 gene (Figure 5). (Emphasis added)

Clearly, Applicants have determined the significance of particular nucleic acid and amino acid changes.

A fourth basis for rejection is that the specification fails to teach *ced-3* genes in any and all organisms. Applicants respectfully disagree. The specification provides methods for the isolation of structurally and functionally related *ced-3* genes. For example, at page 15, lines 21-28, the specification outlines methods to isolate such genes using degenerate oligonucleotides derived from *ced-3*. The specification states that these degenerate oligonucleotides may be used as hybridization probes or as polymerase chain reaction primers. At page 15, lines 29-32, the specification states that polyclonal or monoclonal antibodies may be used as immunoprobes to screen expression libraries for *ced-3* related proteins. At page 16, lines 1-16, the specification discloses methods for identifying *ced-3* related genes in organisms distantly related to *C. elegans*. This strategy involves using "structurally related genes in taxonomically closer organisms as stepping-stones to genes in more distantly related organisms" (page 16, lines 13-15). The

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specification refers to this strategy, at page 16, lines 15 and 16, as "walking along the taxonomic tree." The specification discloses, at page 16, lines 19-25,

> Comparison of members within a gene family, or their encoded products, may indicate functionally important features of the genes or their gene products. Those features that are conserved are likely to be significant for activity. Such conserved sequences can then be used both to identify new members of the gene family...

The specification goes on to distinctly point out functionally significant features of the protein (page 17, lines 1-6),

> Functionally important regions can also be identified by mutagenesis. For example, inactivating mutations of *ced-3* were found to cluster within a region near the COOH-terminus (Figure 5B), suggesting that this region is a functionally important domain of the Ced-3 protein.

Proof of the feasibility of such methods is Applicants' isolation of *ced-3* genes from other organisms (page 59, lines 1-3).

A fifth basis for rejection turns on the assertion that Applicants fail to teach whether Ced-3 related proteins would cause cell death as *C. elegans ced-3* does. The Examiner notes that differences exist between cell death in *C. elegans* and cell death in other organisms, particularly citing a review by Driscoll *et al.*. The Examiner asserts that a pressing issue at the time of filing was whether or not mechanistically similar deaths occur in species other than *C. elegans*.

Applicants teach at page 14, lines 12-20, that differences exist between cell death in vertebrates and cell death in *C. elegans*. Despite these differences, Applicants direct the Examiners attention to the many similarities that exist between cell death in *C. elegans* and cell death in vertebrates. For example, at page 14, lines 20-29, the

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specification teaches that the death of vertebrate and invertebrate cells may be prevented by inhibitors of RNA and protein synthesis. This indicates that both vertebrate and invertebrate cell deaths share a common molecular mechanism, i.e. gene activation.

Furthermore, the specification discloses that the genes induced in both vertebrate and invertebrate dying cells are likely to be similar (page 15, lines 4-15)

> Also supporting the hypothesis that cell death *in C. elegans* is mechanistically similar to cell death in vertebrates is the observation that the protein product of the *C. elegans* gene *ced-9* is similar in sequence to the human protein Bcl-2. *ced-9* has been shown to prevent cells from undergoing programmed cell death during nematode development by antagonizing the activities of *ced-3* and *ced-4* (Hengartner, *et al.*, *Nature 356*: 494-499 (1992)). The *bcl-2* gene has also been implicated in protecting cells against cell death. It seems likely that the genes and proteins with which *ced-9* and *bcl-2* interact are similar as well. (Emphasis added)

Vertebrate *bcl-2* and invertebrate *ced-9* are similar in sequence and in function. This sequence similarity provides further evidence that common molecular mechanisms underlie vertebrate and invertebrate cell death. Furthermore, given the similarities that exist between *bcl-2* and *ced-9*, it seems likely that the Ced-9 interacting protein, Ced-3, would have a counterpart in vertebrates, and that vertebrate Ced-3 would function similarly to *C. elegans ced-3*.

A sixth basis for rejection turns on the assertion that the *C. elegans* bioassay would not be useful for determining the biological activity of Ced-3 related proteins. The Examiner is concerned that an artisan would have to develop a new bioassay to test Ced-3 related proteins. Applicants direct the Examiner's attention to Claim 19, which recites that a *ced-3* related gene complements *ced-3* in the *C. elegans* bioassay. Only a nucleic acid that complements *ced-3* in the *C. elegans* bioassay would be encompassed by the

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claim. Nucleic acids that fail to complement *ced-3* in the *C. elegans* bioassay would not be *ced-3* related genes, and thus would not be encompassed by the claim.

In sum, Applicants have addressed each of the Examiner's concerns. Applicants have shown that *ced-3* related genes and proteins display characteristic *ced-3* structural and functional similarities. Applicants have disclosed methods for the isolation of *ced-3* related genes from organisms other than *C. elegans*. Applicants have demonstrated that common molecular mechanisms exist between vertebrate and invertebrate cell deaths. And, finally, Applicants have disclosed a bioassay that may be used to determine the biological activity of *ced-3* related proteins.

Written Description

Claims 5, 16, and 19 are rejected under 35 U.S.C. § 112, first paragraph. This rejection turns on the assertion that the specification fails to provide a written description supporting Applicants' genus claims to *ced-3* related nucleic acids and proteins.

The first basis for the written description rejection is the assertion that the specification discloses only the *C. elegans ced-3* nucleic acid, SEQ ID NO:18, and encoded protein, SEQ ID NO: 19. This is incorrect. At page 58, lines 34 and 35, and page 59, lines 1-3, Applicants disclose the cloning and sequencing of *ced-3* genes from *C. briggsae* (SEQ ID NO:20) and *C. vulgaris* (SEQ ID NO:21).

The Examiner also asserts that Applicants failed to provide guidance regarding how *ced-3* genes from multiple organisms are related. In fact, Applicants have not only cloned and sequenced multiple *ced-3* genes, but have also provided sequence

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comparisons detailing how the proteins encoded by these nucleic acids are related, for example, at page 58, lines 34 and 35, and at page 59, lines 1-8,

> To identify the functionally important regions of the Ced-3 protein, genomic DNAs containing the *ced-3* genes from two related nematode species, *C. briggsae* and *C. vulgaris*, were cloned and sequenced (Figure 7; SEQ ID NO:20 and 21). Sequence comparison of the three *ced-3* genes showed that the non-serine-rich region of the proteins is highly conserved. In *C. briggsae* and *C. vulgaris*, many amino acids in the serine-rich region are dissimilar compared to the *C. elegans* Ced-3 protein (Figure 7).

The second basis for rejection turns on the assertion that the specification fails to describe relevant identifying characteristics and functional attributes distinguishing members of the claimed genus. The Examiner's attention is once again directed to Figure 7, which details conserved sequences and features. In addition to using sequence comparisons to specifically point out highly conserved regions of the protein, Applicants provide further support for their genus claims by sequencing 12 EMS-induced mutations in the *ced-3* nucleic acid sequence. These *ced-3* mutations are described in Figure 4 and Table 3. Analysis of these mutations identifies highly conserved regions of functional importance, for example, page 17, lines 1-6,

> Functionally important regions can also be identified by mutagenesis. For example, inactivating mutations of *ced-3* were found to cluster within a region near the COOH-terminus (Figure 5B), suggesting that this region is a functionally important domain of the Ced-3 protein. (emphasis added)

The non-serine-rich region is a highly conserved and functionally important region characteristic of all *ced-3* related polypeptides. Mutagenesis analysis also defines characteristic features of Ced-3, for example, at page 59, lines 8-16,

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It seems that what is important in the serine-rich region is the overall serine-rich feature rather than the exact amino acid sequence.
This hypothesis is also supported by analysis of *ced-3* mutations in *C. elegans*: none of the 12 EMS-induced mutations is in the serine-rich region, suggesting that mutations in this region might not affect the function of the Ced-3 protein and thus, could not be isolated in the screen for *ced-3* mutants.

Furthermore, the Applicants have described other characteristics of the Ced-3 protein. For example, Applicants have analyzed the Ced-3 protein's hydrophobicity. Figure 6 is a Kyte-Doolittle hydrophobicity plot of the Ced-3 protein, indicating that the Ced-3 protein (not the *ced-3* nucleic acid) is hydrophilic. This characteristic attribute is significant (page 58, lines 20-22), "The Ced-3 protein is very hydrophilic and no significantly hydrophobic region can be found that might be a trans-membrane domain (Figure 6)." This hydrophilicity is another distinguishing feature of the Ced-3 protein that Applicants would expect all Ced-3 related proteins to share.

In sum, the Applicants have cloned, sequenced, and compared multiple *ced-3* sequences, and have noted distinguishing features and characteristics that define Ced-3-related proteins; therefore, the written description requirement is satisfied, and the written description rejection should be withdrawn.

Rejections under 35 U.S.C. § 112, second paragraph

Claims 5, 6 and 19 are rejected under 35 U.S.C. § 112, second paragraph, for indefiniteness.

Applicants note that Claim 5 has been amended to recite an isolated hydrophilic protein with a serine rich region that functions in cell death. Claim 6 has been amended to recite a Ced-3 protein having the amino acid sequence of SEQ ID NO:19.

The Examiner states that Claim 19 is indefinite because it recites the phrase "structurally related" and "functionally related." The phrase "structurally related" is defined at page 13, lines 15-20,

> Structurally related genes refer herein to genes which have some structural similarity to the nucleotide sequences (genomic or cDNA) of one or both of the *ced-3* or *ced-4* genes, or whose encoded proteins have some similarity to one or both of the amino acid sequences of the Ced-3 or Ced-4 proteins.

The phrase "functionally related" is defined at page 13, lines 20-24,

> Functionally related genes refer to genes, which have similar activity to that of *ced-3* or *ced-4* in that they cause cell death. Such genes can be identified by their ability to complement *ced-3* or *ced-4* mutations in bioassays..."

Clearly, Applicants have defined the phrases "structurally related" and "functionally related." Accordingly, this rejection should be withdrawn.

The Examiner states that Claim 6 is indefinite because figure 4 discloses a nucleic acid sequence as well as a protein sequence. Claim 6 has been amended to recite an isolated polypeptide comprising SEQ ID NO:19.

Rejections under 35 U.S.C. § 102(b) or 103 (a)

Anticipation

Claims 5, 16, and 19 stand rejected under 35 U.S.C. 102(b) as anticipated by or in the alternative under 35 U.S.C. 103(a) as obvious over Yuan (1990). This rejection is respectfully traversed.

Yuan discloses a cosmid, C48D1 and a 12 Kb DNA fragment, C48D10-43, that partially rescues the *ced-3* phenotype when injected into a *ced-3* mutant. Yuan also discloses multiple alleles of *ced-3*.

The Examiner asserts that Claims 5, 16, and 19 are anticipated by Yuan (1990). Applicants respectfully disagree. *Verdegaal Bros. V. Union Oil Co. of California*, 814 F.2d 628, 631, 2USPQ2d 1051, 1053 (Fed. Cir. 1986) "A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Claims 5, 16, and 19 are clearly directed to an isolated protein. Yuan discloses 12 Kb of DNA that partially rescues the *ced-3* phenotype. Clearly Yuan (1990) neither expressly nor inherently describes the isolated Ced-3 protein. The reference fails to clone or describe *ced-3*, to identify the *ced-3* open reading frame, to sequence the *ced-3* encoding nucleic acid, or to identify the amino acid sequence that comprises the Ced-3 protein.

Yuan teaches the general position of *ced-3* on a genetic map. Yuan further discloses fragments of DNA that partially rescue the *ced-3* phenotype. This is not sufficient to anticipate the invention. The invention is directed to a family of structurally

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and functionally related Ced-3 polypeptides. These peptides are hydrophilic in nature and comprise a serine-rich region. None of this information is expressly or inherently present in the 12 Kb piece of DNA isolated by Yuan. Accordingly, this rejection may be withdrawn.

Obviousness

Claims 5, 16, and 19 are rejected under 35 U.S.C. 102(b) as obvious over Yuan (1990).

The Examiner asserts that,

> ...the encoded protein will be considered an inherent property of the ced-nucleic acids of Yuan. Thus, the claimed invention as a whole was at least prima facie obvious over, if not anticipated by, the prior art. (page 12, lines 10-12)

Applicants direct the Examiner's attention to the three basic criteria that must be met to establish a *prima facie* case of obviousness. MPEP § 2142 states,

> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second there must be a reasonable expectation of success. Finally, the prior art reference(or references when combined) must teach or suggest all the claim limitations.

Claims 5, 16, and 19 are directed to an isolated protein. Furthermore, amended claim 19 recites a polypeptide that is hydrophilic in nature and has a serine rich region. Yuan (1990) teaches only a 12 Kb piece of DNA.

> ...*ced-3* has now been localized to a fragment of DNA about 12 Kb long. Further experiments are needed to define the position of *ced-3* more precisely and to

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analyze this gene molecularly. (Yuan (1990) page 213, lines 5-8) (Emphasis added)

Furthermore, Yuan (page 213, lines 22-25) discloses that the cosmid, C48D1, from which the 12 Kb fragment was subcloned may not contain all of *ced-3*, because none of Yuan's tranformants exhibited complete rescue of the *ced-3* mutant phenotype. Therefore, Yuan reasons, a part of the *ced-3* gene might be missing from the cosmid. Yuan states (page 213, lines 22-25), "A small part of the *ced-3* gene (regulatory elements or a part of the coding region) may be missing from the cosmid C48D1." Not only does Yuan fail to disclose a Ced-3 protein that would teach or suggest all of the limitations of Claims 5, 16 and 19, but Yuan also acknowledges that the DNA used to transform the *ced-3* mutants may not contain all of *ced-3*.

Even if Yuan identified a fragment of DNA containing the entire *ced-3* gene, this would not have been sufficient to meet the criterion defined for obviousness. The instant invention is directed to a newly discovered family of *ced-3* related proteins. These proteins are defined by their structural and functional relatedness. Yuan fails to teach at least three important aspects of the present invention: the cloning of a *ced-3* gene, the nucleotide sequence of a *ced-3* gene, and the amino acid sequence of a Ced-3 protein. Yuan merely identifies a large fragment of DNA that partially rescues *ced-3*. This piece of DNA may, or may not, comprise the complete *ced-3* open reading frame.

In sum, the present invention is directed to a family of Ced-3 polypeptides that are structurally related. The invention features Ced-3 polypeptides that are hydrophilic in nature and contain a serine rich region. Moreover, Applicants describe the isolation of

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two additional Ced-3 family members, and compare the sequence of three Ced-3 proteins. This sequence comparison allows Applicants to define conserved amino acid sequences that are characteristic of Ced-3 polypeptides. In addition, Applicants sequenced twelve alleles of Ced-3, and identified regions of functional importance. The identification of conserved amino acid regions of functional importance is critical to the isolation of additional Ced-3 family members. None of these features of the instant invention is obvious over Yuan (1990). The rejection for obviousness should be withdrawn.

PTO 1449

Applicants note that the forms PTO 1449 submitted with the Information Disclosure Statements filed September 14, 2000 have not been initialled and returned and hereby request that they be initialled and returned.

Respectfully submitted,

Date: January 16, 2002

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Version With Markings to Show Changes

5. (Twice Amended) An isolated protein encoded by a *ced-3* nucleic acid, said protein being hydrophilic in nature, comprising a serine-rich region, and functioning in cell death.

6. (Twice Amended) An isolated protein having the amino acid sequence of [Figure 4] SEQ ID NO.:19.

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Pending Claims

5. (Twice Amended) An isolated protein encoded by a *ced-3* nucleic acid, said protein being hydrophilic in nature, comprising a serine-rich region, and functioning in cell death.

6. (Twice Amended) An isolated protein having the amino acid sequence of SEQ ID NO.:19.

16. An isolated protein encoded by a *ced-3* nucleic acid comprising a mutation.

19. An isolated protein encoded by an isolated nucleic acid comprising:

(a) a nucleic acid which is structurally related to the *ced-3* nucleic acid sequence of SEQ ID NO:18, wherein the polypeptide encoded by said nucleic acid is hydrophilic in nature and has a serine rich region;

(b) a nucleic acid which is functionally related to the *ced-3* nucleic acid, wherein said functionally related nucleic acid encodes a protein that causes cell death, wherein cell death is measured by the ability of said nucleic acid to complement *ced-3* or *ced-4* mutations in an *in vivo* or *in vitro* bioassay; and

(c) a nucleic acid which is both structurally and functionally related to the *ced-3* nucleic acid as described in (a) and (b).

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